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(71) Applicant: **THE UPJOHN COMPANY**  
301 Henrietta Street  
Kalamazoo, Michigan 49001(US)

(72) Inventor: **Dugaiczky, Achilles**  
c/o The Upjohn Company 301 Henrietta Street  
Kalamazoo Michigan 49001(US)

(74) Representative: **Perry, Robert Edward et al,**  
**GILL JENNINGS & EVERY 53-64 Chancery Lane**  
London WC2A 1HN(GB)

(54) **Albumin-based nucleotides, their replication and use, and plasmids for use therein.**

(57) The DNA sequence coding for human serum albumin has been isolated and inserted as two fragments into two novel plasmids which can be replicated in *E. coli*. These novel fragments can be joined to provide a unitary DNA sequence which then can be cloned into a suitable host, e.g. *E. coli*, for the expression of human serum albumin (which is used extensively in medical practice in treating shock conditions).

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ALBUMIN-BASED NUCLEOTIDES, THEIR REPLICATION  
AND USE, AND PLASMIDS FOR USE THEREIN

This invention relates to nucleotides related to human serum albumin (HSA), their replication and use, and plasmids (and host substances) for use therein.

The gene for serum albumin is regulated in development. On the other hand, serum albumin is synthesised in mammals by the adult liver, and its plateau in adulthood. The embryonic liver and yolk sac, on the other hand, produce predominantly  $\alpha$ -fetoprotein, but the synthesis decreases drastically after birth. Recently, Law et al determined the complete sequence of mouse  $\alpha$ -fetoprotein mRNA, Nature 291 (1981) 201-205. The structure revealed extensive homology to mammalian serum albumin, indicating that the two proteins are encoded in the same gene family. Similar conclusions have been reached from studies on the  $\alpha$ -fetoprotein genes of the rat and the mouse; see Jagodzinski et al, Proc. Natl. Acad. Sci. USA, 78 (1981) 3521-3525, and Gorin et al, J. Biol. Chem. 256 (1981) 1954-1959.

The complete nucleotide sequence of human serum mRNA has been determined from recombinant cDNA clones and from a primer-extended cDNA synthesis on the mRNA template. The sequence comprises 2,078 nucleotides, starting upstream of a potential ribosome binding site in the 5'-untranslated region. It contains all the translated codons and extends into the poly(A) at the 3'-terminus. Part of the translated sequence codes for a hydrophobic prepeptide met-lys-trp-val-thr-phe-ile-ser-leu-leu-phe-leu-phe-ser-ser-ala-tyr-ser, followed by a basic propeptide arg-gly-val-phe-arg-arg. These signal peptides are absent from mature serum albumin and, so far, have not been identified in their nascent state in humans. A remaining 1,755 nucleotides of the translated mRNA sequence code for 585 amino acids which are in agreement, with few exceptions, with the published amino acid data for human serum albumin. The mRNA sequence verifies and refines the repeating homology in the triple-domain structure of the serum albumin molecule.

DETAILED DESCRIPTION OF THE INVENTION

Human serum albumin cDNA is cloned into the PstI site of plasmid pBR322 by the oligo(dG)-oligo(dC) tailing technique. Plasmid DNA was isolated from 97 positive colonies which hybridized to the enriched albumin cDNA probe, and the recombinant plasmid pHA36 was found to contain the largest insert of an albumin cDNA sequence. Its restriction endonuclease map is shown in the drawing, together with a restriction map of the primer-extended plasmid clone pHA206. The latter was obtained in a second transformation experiment after initiating the cDNA synthesis from an internal primer. This primer was a 91 base pairs long DNA fragment, MspI(152)-TaqI(182/3), isolated from pHA36. The two plasmids, pHA36 and pHA206, share 0.15 kb of homologous DNA. Together, they encode the entire sequence for human serum albumin, starting with the CTT codon for leu -10 of the prepeptide and extending into the 3'-untranslated region of poly(A).

Sequence of the Albumin cDNA. The sequence was determined for the most part on both DNA strands to ensure accuracy. All of the restriction sites used to end-label DNA fragments were sequenced across by labeling a neighboring restriction site. The entire nucleotide sequence of the serum albumin mRNA, as determined from the cloned DNA in pHA36, pHA206, and from the primer-extended cDNA at the 5'-terminus of the message, is shown in the following Table 1. The inferred amino acid sequence is also indicated. The mRNA length is 2,078 nucleotides, of which 38 represent the 5'-untranslated region, 54 identify a prepeptide of 18 amino acids, 18 identify a propeptide of 6 amino acids, 1,755 code for the known 585 amino acids of serum albumin, 189 make up the 3'-untranslated region and 24 are the poly(A) sequence. Nucleotides 5 to 15 (-34 to -24) in the 5'-untranslated region (Table 1) are complementary to a 3'-terminal region of eukaryotic 18S RNA [Azad, A.A. and Deacon, N.J. (1980) Nucl. Acids Res. 8, 4365-4376] and thus could represent a ribosome binding site:

(5')...T T<sup>C</sup> T C T T C T G T.....albumin mRNA  
 (3')...G A G G A A G G C G U C C m<sub>2</sub>A<sup>6</sup> m<sub>2</sub>A<sup>6</sup>.....18S RNA

The translated portion of the mRNA sequence codes for the signal peptide and the main body of the albumin polypeptide chain. The

signal peptide is composed of a hydrophobic prepeptide of 18 amino acids and a basic propeptide of 6 amino acids (Table 1). Since pre-peptides are removed from nascent secretory proteins (like albumin) in the endoplasmic reticulum, they are seen only in vitro in heterologous  
5 translation systems. As yet, they have not been found within cells [Judah, J.D. and Quinn, P.S. (1977) FEBS 11th Mtg., Copenhagen 50, 21-29; and Strauss, A.W., Donohue, A.M., Bennett, C.D., Rodkey, J.A. and Alberts, A.W. (1977) Proc. Natl. Acad. Sci. USA 74, 1358-1362]. This is the first report of the presence and the sequence of a pre-  
10 peptide for human serum albumin. As it is with other secretory proteins, the conversion of proalbumin to albumin takes place in the Golgi vesicles, and the enzyme responsible for this cleavage is probably cathepsin B [Judah, J.D. and Quinn, P.S. (1978) Nature 271, 384-385]. This is also a first report on the sequence of the pro-  
15 peptide for normal human serum albumin.

At the 3'-end of the message, the putative polyadenylation signal sequence, AATAAA, is located 164 nucleotides downstream from the amino acid termination codon TAA and 16 nucleotides upstream from the beginning of the poly(A) sequence. Another characteristic sequence  
20 located near the polyadenylation site has been identified by Benoist, et al. [Benoist, C., O'Hare, K., Breathnach, R. and Chambon, P. (1980) Nucl. Acids Res. 8, 127-142]; the consensus sequence from several mRNAs was concluded as TTTTCACTGC. A similar sequence, TTTTCTCTGT, is located 19 nucleotides upstream from the AATAAA hexanucleotide in the  
25 human albumin mRNA (Table 1).

30

35

	-18	p r o	-10	
		Met lys trp val tlu phe lle ser leu leu phe leu phe ser		
		GCTTTTCTTCTGTCAACCCACAGCCTTTGCACA ATG AAG TGG GTA ACC TTT ATT TCC CTT CTT TTT CTC TTT AGC	(30)	
	-1	-6	p r o	-1
		ser ala tyr ser arg gly val phe arg arg asp ala his lys ser glu val ala his asp phe lys asp leu qly qlu glu asn phe lys	20	
		TCC GCT TAT TCC AGC CGT GTG TTT CGT CGA CAT GCA CAC AAC ACT GAG GTT GCT CAT CGG TTT AAA GAT TTG GCA GAA GAA AAT TTC AAA	(170)	
21			34	40
		ala leu val leu lle ala phe ala gln tyr leu gln cys pro phe qlu asp his val lys leu val asn glu val thr qlu phe ala	50	
		GCC TTG GTG TTG ATT GCC TTT GCT TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA CAT CAT GTA AAA TTA GTG AAT GAA GTA ACT CAA TTT GCA	(260)	
51	53		60	62
		lys thr cys val ala asp glu ser ala glu asn cys asp lys ser leu his thr leu phe qly asp lys leu cys thr val ala thr leu	80	
		AAA ACA TGT GTT GCT CAT CAG TCA GCT GA AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT CGA GAC AAA TTA TGC ACA GTT GCA ACT CTT	(350)	
81			90	91
		arg glu thr tyr gly glu met ala asp cys cys ala lys gln glu pro qly arg asn qlu cys phe leu qln his lys asp asp asn pro	110	
		CCGT GAA ACC TAT GCT GAA ATG GCT CAC TGC TGT GCA AAA CAA GAA CCT CGG ACA AAT GAA TGC TTC TTC CAA CAC AAA GAT GAC AAC CCA	(440)	
111			120	124
		asn leu pro arg leu val arg pro glu val asp val met cys thr ala phe his asp asn qlu thr phe leu lys lys tyr leu try	140	
		AAC CTC CCC CCA TTG GTG ACA CCA GAG GTT GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTT TTG AAA AAA TAC TTA TAT	(330)	
141			150	160
		lle ala arg arg his pro tyr phe tyr ala pro glu leu leu phe phe ala lys arg tyr lys ala ala phe thr qlu cys qln	168	169
		GAA ATT GCC ACA AGA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA	(620)	
171			177	180
		ala asp lys ala ala cys leu leu pro lys leu asp glu leu arg asp qlu qly lys ala ser ser ala lys qln arg leu lys cys	200	
		GCT GCT GAT AAA CCT GCC TGC CTG TTG CCA AAG CTC GAT GAA CTT CGG CAT GAA CGG AAG GCT TCG TCT GCC AAA CAG ACA CTC AAG TGT	(710)	
201			210	220
		ala ser leu gln lys phe gly glu arg ala phe lys ala trp ala val ala arg leu ser gln arg phe pro lys ala qlu phe ala qlu	230	
		CCC AGT CTC CAA AAA TTT GGA GAA ACA GCT TTC AAA GCA TGG CCA GTA GCT CCC CTG AGC CAG ACA TTT CCC AAA GCT GAG TTT GCA GAA	(300)	

231 240 245 246 250 253 260  
 val ser lys leu val thr asp leu thr lys val his thr glu cys cys his gly asp leu leu glu cys ala asp asp arg ala asp leu  
 GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA GTC CAC ACC GAA TGC TCC CAT GCA CAT CTG CTT GAA TGT GGT CAT CAC ACG CCG CAC CTT (890)

261 265 270 278 279 280 289 290  
 ala lys tyr ile cys glu asn gln asp ser ile ser ser lys leu lys glu cys cys glu lys pro leu leu glu lys ser his cys ile  
 CCC AAG TAT ATC TGT GAA AAT CAA GAT TCG ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA AAA CCT CTG TTG CAA AAA TCC CAC TCC ATT (980)

291 300 310 316 320  
 ala glu val glu asn asp glu met pro ala asp leu pro ser leu ala ala asp phe val glu ser lys asp val cys lys asn tyr ala  
 CCC GAA GTG CAA AAT GAT GAG ATG CCT GCT CAC TTG CCT TCA TTA GCT GCT CAT TTT CTT GAA AGT AAG CAT GTT TGC AAA AAC TAT GGT (1070)

321 330 340 350  
 glu ala lys asp val phe leu gly met phe leu tyr glu tyr ala arg arg his pro asp tyr ser val val leu leu leu arg leu ala  
 CAG GCA AAG GAT GTC TTC TTG GCC ATG TTT TTG TAT GAA TAT GCA AGA AGC CAT CCT CAT TAC TCT GTC GTC CTG CAG CTT GCC (1160)

351 360 361 369 370 380  
 lys thr tyr glu thr thr leu glu lys cys cys ala ala asp pro his glu cys tyr ala lys val phe asp glu phe lys pro leu  
 AAG ACA TAT GAA ACC ACT CTA GAG AAG TCC TGT GCT GCT CCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC CAT GAA TTT AAA CCT CTT (1250)

381 390 392 400 410  
 val glu glu pro gln asn leu ile lys gln asn cys glu leu phe glu qln leu qly glu tyr lys phe qln asn ala leu leu val arg  
 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAT TGT GAG CTT TTT CAG CAG CTT GCA GAG TAC AAA TTC CAG AAT GCG CTG TTA GTT CGT (1340)

411 420 430 437 438 440  
 tyr thr lys lys val pro gln val ser thr pro thr leu val glu val ser arg asn leu qly lys val qly ser lys cys cys lys his  
 TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT CCA ACT CTT GTA CAG GTC TCA ACA AAC CTA GCA AAA GTG GCG AGC AAA TGT TGT AAA CAT (1430)

441 448 450 460 461 470  
 pro glu ala lys arg met pro cys ala glu asp tyr leu ser val val leu asn gln leu cys val leu his glu lys thr pro val ser  
 CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTG AAC CAG TTA TGT GTG TTG CAT GAG AAA ACC CCA GTA AGT (1520)

471 476 477 480 490 500  
 asp arg val thr lys cys cys thr glu ser leu val asn arg arg pro cys phe ser ala leu glu val asp glu thr tyr val pro lys  
 GAC AGA GTC ACC AAA TCC TCC ACA GAA TCC TTG GTC AAC AGG CCA TCC TTT TCA CCT CTG GAA GTC CAT GAA ACA TAC GTT CCC AAA (1610)

501 510 514 520 530  
 glu phe asn ala glu thr phe thr phe his ala asp ile cys thr leu ser glu lys arg qln ile lys lys qln thr ala leu val  
 CAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TCC ACA CTT TCT CAG AAG GAG AGA CAA ATC AAG AAA CAA ACT CCA CTT GTT (1700)

35 531 540 550 558 559 560  
glu leu val lys his lys pro lys ala thr lys glu gln leu lys ala val met asp phe ala ala phe val glu lys cys lys  
GAG CTC GTG AAA CAC AAG CCC AAG GCA ACA AAA GAG CAA CTG AAA CCT GTT ATG GAT TTC GCT TTT GTA GAG AAG TGC AAG (1790)

561 567 570 580  
ala asp asp lys glu thr cys phe ala glu glu gln lys lys leu val ala ala ser gln ala leu gln leu ter  
GCT GAC GAT AAG GAG ACC TGC TTT GCC GAG GAG GGT AAA AA CTT GTT GCT GCA AGT CAA GCT GCC TTA TAA CATCACATTTAAAG (1883)

ter ter  
CATCTCAGCCTACCATGAGATACAGAGAAAGATGAGATGCTTATTCATCTGTTTTCTTTTCGTTGGTGTAAAGCCACACCCCTGCTCTAAAAACATAAATTTCTTTAA (2002)

TCATTTGGCCTCTTTTCTCTGCTTCAATTAATAAAATGCAAGATCTAA..... 20 .....AA (2078)

Following are examples which illustrate procedures, ~~including the best mode~~, for practicing the invention. These examples should not be construed as limiting. All percentages are by weight and all solvent mixture proportions are by volume unless otherwise noted.

5 Example 1      Isolation of Messenger RNA

Human liver mRNA was obtained following the procedure of Chirgwin, et al [Chirgwin, J.M., Przybyla, A.E., MacDonald, R.J. and Rutter, W.J. (1979) Biochemistry 18, 5294-5299]. Immunoprecipitation of albumin containing polysomes was performed according to Taylor and  
10 Tse [Taylor, J.M. and Tse, T.P.H. (1976) J. Biol. Chem. 251, 7461-7467]. In vitro translation of mRNA was carried out in a reticulocyte cell-free system, following the instruction of the manufacturer (New England Nuclear). The translation products were separated electrophoretically according to Laemmli [Laemmli, J.K. (1970) Nature 227,  
15 680-685.

Example 2      Cloning Procedures

Double stranded cDNA was synthesized as described previously [Law, S., Tamaoki, T., Kreuzaler, F. and Dugaiczyk, A. (1980) Gene 10, 53-61]. It was annealed to PstI-linearized pBR322 DNA [Bolivar, F.,  
20 Rodriguez, R.L., Greene, P.J., Betlach, M.C., Heyneker, H.L., Boyer, H.W., Crossa, J.H. and Falkow, S. (1977) Gene 2, 95-113] that had been tailed with 15 dG residues/3'-terminus [Dugaiczyk, A., Robberson, D.L. and Ullrich, A. (1980) Biochemistry 19, 5869-5873]. The annealed DNA was used to transform E. coli strain RR1, as detailed previously [Law, S., et al., Ibid.]. The albumin clones were selected using the colony  
25 hybridization method of Grunstein and Hogness [Grunstein, M. and Hogness, D.S. (1975) Proc. Natl. Acad. Sci. USA 72, 3961-3965], with [<sup>32</sup>P]-labeled cDNA synthesized with the immunoprecipitated polysomal mRNA as template.

30 As shown in Example 5, plasmids pHA36 and pHA206 were deposited in E. coli HB101 hosts. The plasmids were obtained from E. coli RR1 hosts, described in this example, and transformed into E. coli HR101 by standard procedures well known to those of ordinary skill in this art. The E. coli RR1 hosts were lysed and then centrifuged to  
35 separate the chromosomal DNA, cell DNA and plasmid DNA. The plasmid DNA, remaining in the supernatant, is precipitated with ethanol and the precipitate is resuspended in buffer, e.g., TCM (10mM Tris·HCl, pH 8.0, 10 mM CaCl<sub>2</sub>, 10 mM MgCl<sub>2</sub>). The cells for transformation are



prepared as follows: 120 ml of L-broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) are inoculated with an 18 hour culture of HB101 NRRL B-11371 and grown to an optical density of 0.6 at 600 nm. Cells are washed in cold 100 mM NaCl and resuspended for 15 minutes in 20 ml chilled 50 mM CaCl<sub>2</sub>. Bacteria are then concentrated to one-tenth of this volume in CaCl<sub>2</sub> and mixed 2:1 (v:v) with annealed plasmid DNA, prepared as described above. After chilling the cell-DNA mixture for 15 minutes, it is heat shocked at 42°C for 2 minutes, then allowed to equilibrate at room temperature for ten minutes before addition of L-broth 10 times the volume of the cell-DNA suspension. Transformed cells are incubated in broth at 37°C for one hour before inoculating selective media (L-agar plus 10 µg/ml tetracycline) with 200 µl/plate. Plates are incubated at 37°C for 48 hours to allow the growth of transformants.

15 Example 3      Mapping of Restriction Endonuclease Sites

Restriction endonucleases were obtained from Bethesda Research Laboratories and New England Biolabs and were used according to the manufacturers' instructions. The digested DNA fragments were analyzed electrophoretically on agarose [Helling, R.R., Goodman, H.M. and Boyer, H.W. (1974) J. Virol. 14, 1235-1244] or acrylamide [Dingman, C., Fisher, M.P. and Kakefuda, T. (1972) Biochemistry 11, 1242-1250] gels.

20 Example 4      DNA Sequencing

DNA fragments were dephosphorylated with bacterial alkaline phosphatase (Worthington) and labeled at the 5'-ends with polynucleotide kinase (Boehringer-Mannheim) and  $\gamma$ [<sup>32</sup>P]ATP. Following digestion with a second restriction endonuclease and electrophoretic separation of the fragments, DNA sequence determination was done according to the procedure of Maxam and Gilbert [Maxam, A. and Gilbert, W. (1980) Methods Enzym. 65, 499-560] and the degradation products were separated electrophoretically on 0.4 mm acrylamide gels as described by Sanger and Coulson [Sanger, F. and Coulson, R. (1978) FEBS Letters 87, 107-110].

30 Example 5      Recombinant Plasmids pHA36 and pHA206

As disclosed in Example 2, albumin clones were selected by hybridizing to the enriched albumin cDNA probe. Plasmid pHA36 contained the largest insert of an albumin cDNA sequence. Both plasmids pHA36 and pHA206 have been deposited in a viable E. coli host in the

permanent collection of the Northern Regional Research Laboratory (NRRL), U.S. Department of Agriculture, Peoria, Illinois, U.S.A. Their accession numbers in this repository are as follows:

HB101(pHA36) - NRRL B-12551

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HB101(pHA206) - NRRL B-12550

E. coli HB101 is a known and widely available host microbe. Its NRRL accession number is NRRL B-11371.

NRRL B-12550 and NRRL B-12551 are available to the public, ~~upon the grant of a patent. It should be understood that the availability~~  
10 of these deposits does not constitute a license to practice the subject invention in derogation of patent rights granted with the subject instrument by governmental action.

E. coli RR1 and E. coli HB101 are known and widely available host microbes. Their NRRL accession numbers are NRRL B-12186 and NRRL  
15 B-11371, respectively.

pBR322 is a well known and widely available plasmid. It can be obtained from the following host deposit by standard procedures:

NRRL B-12014 - E. coli RR1 (pBR322).

YEp6 is a well known and widely available yeast episomal plasmid.  
20 It can be obtained from the following host deposit by standard procedures:

E. coli HB101 (YEp6) - NRRL B-12093.

Example 6 Assembly of the Serum Albumin Gene

Assembling the pieces together is a straightforward task of re-  
25 striction enzymology. There is only one MspI site in the overlapping DNA sequence of the two cDNA clones. Two enzymatic steps of (i) MspI digestion of the two DNAs, followed by (ii) the use of ligase, an enzyme that seals DNA fragments, will give the desired product. Although two other undesired DNA species will also be obtained in the  
30 course of this recombination reaction, both of them will differ substantially in size. Thus, separation and isolation of the desired DNA species will be achieved.

The assembled DNA clone can be used to transform two types of cells:

- 35 (a) Escherichia coli  
(b) Saccharomyces cerevisiae

- (a) The vector of choice is plasmid pBR322, the same that has

been successfully used for cloning of the two fragmented pieces of the serum albumin cDNA.

(b) In order to transform yeast with the serum albumin structural gene sequence, the DNA must be inserted into one of the existing yeast plasmid vectors. This can be accomplished by taking advantage of the fact that several restriction endonuclease recognition sequences are absent from the cloned serum albumin DNA. Synthetic EcoRI DNA linkers can be ligated to the DNA fragment containing the serum albumin sequence followed by insertion (ligation) into one of the yeast plasmid vectors, e.g., YEp6, at the Eco RI cloning site. The fused chimeric plasmid can be used to transform yeast according to an established procedure [Hinnen, A., Hicks, J.R. and Fink, G.R. (1978) Proc. Natl. Acad. Sci. USA, 75, 1929]. YEp6 can be obtained from the NRRL repository, as disclosed supra.

15 Example 7      Expression of the Serum Albumin Gene

The main body of the structural gene will be transcribed by the E. coli or yeast enzymes. If little or no albumin is produced with the selected host, then an Escherichia coli promoter DNA sequence carrying an initiation codon, i.e., ATG, can be ligated at the beginning of the serum albumin structural gene. Such elements are known and available, e.g., lac promoter used for the expression of human interferon gene in E. coli [Proc. Natl. Acad. Sci. 77, 5230 (1980)]; source of promoter DNA [Proc. Natl. Acad. Sci. 76, 760 (1979)]. Also, see Nature, Vol. 281, October 18, 1979. It has already been documented that such Escherichia coli promoter sequences function well in the expression of foreign genes in Escherichia coli [Mercereau-Puijalon, O., Royal, A., Cami, B., Garapin, A., Krust, A., Gannon, I. and Kourilsky, P. (1978) Nature 275, 505; and Goeddel, D.V., Kleid, D.G., Bolivar, F., Heyneker, H.L., Yansura, D.G., Grea, R., Hirose, T., Kraszewski, A., Itakura, K., and Riggs, A. (1979) Natl. Acad. Sci. USA 76, 106]. For expression in yeast, see Rose, M., Casadaban, M.J. and Botstein, D. (1981) Proc. Natl. Acad. Sci. USA 78, 2460 and 4466.

30 Example 8      Screening of Clones Producing Albumin

Immunological methods can be used to detect small amounts of albumin made in a bacterium. Flat disks of flexible polyvinyl are coated with the IgG fraction from an immune serum and the disks are pressed onto an agar plate so that antigen released from an in situ lysed microbial colony can bind to the fixed antibody. The plastic

disk is then incubated with the same total IgG fraction labeled with radioactive iodine so that other determinants on the bound antigen can in turn bind the iodinated antibody. Radioactive areas on the disk expose X-ray film during autoradiography and thus identify colonies  
5 producing the protein which is being screened for. Detailed protocols of this procedure have been published [Broome, S. and Gilbert, W. (1978) Proc. Natl. Acad. Sci. USA, 75, 2746]. The purification of human serum albumin can be accomplished by using procedures well known in the art. For example, procedures disclosed in a chapter by T.  
10 Peters: Purification and Properties of Serum Albumin, in: The Plasma Proteins, Putnam, Ed. Academic Press, New York, 1975, can be used.

The work described herein was all done in conformity with physical and biological containment requirements specified in the NIH Guidelines.

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CLAIMS

1. Plasmid pHA36, having a restriction endonuclease pattern as shown in the drawing.

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2. Plasmid pHA206, having a restriction endonuclease pattern as shown in the drawing.

3. E. coli HB101 (pHA36) having the deposit accession number  
10 NRRL B-12551.

4. E. coli HB101 (pHA206) having the deposit accession number  
NRRL B-12550.

15 5. A microorganism modified to contain a nucleotide sequence coding for the amino acid sequence of human serum albumin; said nucleotide sequence is as follows:

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35                   -1   -6   p   r   o   -1   1                   10                   20                   50  
                   ser ala tyr ser arg gly val phe arg arg asp ala his lys ser glu val ala his arg phe lys asp leu gln glu asn phe lys                   leu phe leu phe ser  
                   TCG CCT TAT TCC ACC GGT GTG TTT CGT CCA GAT GCA CAC AAC AGT CAG GTT GCT CAT CGC TTT AAA CAT TTG GCA GAA GAA AAT TTC AAA                   CTT TTT CTC TTT ACC (30)  
  
                   21                   30                   34                   40                   50  
                   ala leu val leu ile ala phe ala gln tyr leu gln gln cys pro phe glu asp his val lys leu val asn glu val thr gln phe ala  
                   GCC TTG GTG TTG ATT GCC TTT CCT CAG TAT CTT CAG CAG TGT CCA TTT GAA CAT CAT GTA AAA TTA GTG AAT CAA GTA ACT CAA TTT GCA (260)  
  
                   51                   53                   60                   62                   70                   75                   80  
                   lys thr cys val ala asp glu ser ala glu asn cys asp lys ser leu his thr leu phe gln phe lys leu cys thr val ala thr leu  
                   AAA ACA TGT GTT GCT CAT CAG TCA CCT GAA AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT CCA GAC AAA TTA TGC ACA GTT GCA ACT CTT (350)  
  
                   81                   90                   91                   100                   101                   110  
                   arg glu thr tyr gly glu met ala asp cys cys ala lys gln glu pro gly arg asn glu cys phe leu gln his lys asp asp asn pro  
                   CGT GAA ACC TAT GGT GAA ATG CCT GAC TGC TGT GCA AAA CAA GAA CCT GCG ACA AAT CAA TGC TTC CAA CAC AAA GAT GAC AAC CCA (400)  
  
                   111                   120                   124                   130                   140                   140  
                   asn leu pro arg leu val arg pro glu val asp val met cys thr ala phe his asp asn glu thr phe leu lys lys tyr leu try  
                   AAC CTC CCC CCA TTG GTG ACA CCA CAG GTT GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA CAG ACA TTT TTG AAA AAA TAC TTA TAT (330)  
  
                   141                   150                   160                   168                   169                   170  
                   glu ile ala arg arg his pro tyr phe tyr ala pro glu leu leu phe phe ala lys arg tyr lys ala ala phe thr gln cys cys gln  
                   CAA ATT GCC ACA ACA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG TAT AAA GCT GCT TTT ACA GAA TGT TGC CAA (420)  
  
                   171                   177                   180                   190                   200  
                   ala ala asp lys ala ala cys leu leu pro lys leu asp glu leu arg asp glu gln lys ala ser ser ala lys gln arg leu lys cys  
                   CCT GCT CAT AA GCT GCC TGC CTG TTG CCA AAC CTC GAT GAA CCG MAG GCT TCG TCT GCC AAA CAG ACA CTC MAG TGT (710)  
  
                   201                   210                   220                   230  
                   ala ser leu gln lys phe gly glu arg ala phe lys ala trp ala val ala arg leu ser gln arg phe pro lys ala gln phe ala gln  
                   CCC AGT CTC CAA AAA TTT GCA GAA ACA GCT TTC AAA CCA TCG CCA GTA CCT CCC CTG ACC CAG ACA TTT CCC AAA GCT GAG TTT GCA CAA (300)

231 260 245 246 250 253 260  
 val ser lys leu val thr asp leu thr lys val his thr glu cys cys his gly asp leu leu glu cys ala asp asp arg ala asp leu  
 GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA GTC CAC AGG GAA TGC CAT GCA GAT CTG CTT GAA TGT GCT GAT GAC AGG GCG GAC CTT (890)

261 265 270 278 279 280 289 290  
 ala lys tyr ile cys glu asn gln asp ser ile ser ser lys leu lys glu cys cys glu lys pro leu leu glu lys ser his cys ile  
 CCC AAG TAT ATC TGT GAA AAT CAA CAT TCC ATC TCC AGT AAA CTG AAG GAA TGC TGT GAA AAA CCT CTG TTG GAA AAA TCC CAC TCC ATT (980)

291 300 310 316 320  
 ala glu val glu asn asp glu met pro ala asp leu pro ser leu ala ala asp phe val glu ser lys asp val cys lys asn tyr ala  
 GCC GAA GTG CAA AAT CAT CAG ATG CCT GCT CAC TTG CCT TCA TTA GCT GCT GAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT GCT (1070)

321 330 340 350  
 glu ala lys asp val phe leu gly met phe leu tyr glu tyr ala arg arg his pro asp tyr ser val val leu leu leu arg leu ala  
 CAG GCA AAG GAT GTC TTC TTG GCC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT TAC TCT GTC CTG CTG CTG ACA CTT GCC (1160)

351 360 361 369 370 380  
 lys thr tyr glu thr thr leu glu lys cys cys ala ala asp pro his glu cys tyr ala lys val phe asp glu phe lys pro leu  
 AAG ACA TAT GAA ACC ACT CTA GAG AAG TGC TGT GCC GCT GCA GAT CCT CAT GAA TGC TAT GCC AAA GTG TTC CAT GAA TTT AAA CCT CCT (1250)

381 390 392 400 410  
 val glu glu pro gln asn leu ile lys gln asn cys glu leu phe glu leu phe gln asn ala leu leu val arg  
 CTC GAA GAG CCT CAG AAT TTA ATC AAA CAA AAT TGT GAG CTT TTT GAG CAG CTT GCA GAG TAC AAA TTC CAG AAT GCG CTG TTA CTT CGT (1340)

411 420 430 437 438 440  
 tyr thr lys lys val pro gln val ser thr pro thr leu val glu val ser arg asn leu qly lys val qly ser lys cys cys lys his  
 TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GCA AAA GTG GCG AGC AAA TGT TGT AAA CAT (1430)

441 448 450 460 461 470  
 pro glu ala lys arg met pro cys ala glu asp tyr leu ser val val leu asn gln leu cys val leu his glu lys thr pro val ser  
 CCT GAA GCA AAA ACA ATG CCC TGT GCA GAA CAC TAT CTA TCC GTC GTC CAC TTA TGT GTG TTG CAT CAG AAA ACG CCA GTA AGT (1520)

471 476 477 480 490 500  
 asp arg val thr lys cys cys thr glu ser leu val asn arg arg pro cys phe ser ala leu glu val asp glu thr tyr val pro lys  
 CAC ACA GTC ACC AAA TGC TCC ACA GAA TCC TTG GTG AAC AGG CCA CCA TGC TTT TCA GCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA (1610)

501 510 514 520 530  
 glu phe asn ala glu thr phe thr phe his ala asp ile cys thr leu ser glu lys glu arg gln ile lys lys gln thr ala leu val  
 CAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TCC ACA CTT TCT CAG AAG CAG ACA CAA ATC AAG AAA CAA ACT GCA CTT GTT (1700)

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1  
10  
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531  
glu leu val lys hls lys pro lys ala thr lys glu gln leu lys ala val met asp phe ala ala phe val glu lys cys lys  
GAG CTC GTG AAA CAC AAG CCC AAG GCA ACA AAA GAG CAA CTG AAA GCT GTT ATG CAT CAT TTC GCT TTT GTA GAG AAG TCC TCG AAG (1790)

540  
550  
558 559 560

561  
ala asp asp lys glu thr cys phe ala glu glu gln lys lys leu val ala ala ser gln ala ala leu gly leu ter  
GCT GAC GAT AAG GAG ACC TGC TTT CCC GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA GCT GCC TTA GGC TTA TAA CATCACATTAAAG (1883)

567  
570  
580

ter ter

CATCTCAGCCTACCATGACATAAGACAGAAAGAAATGAGATCAAAAGCTTATTCATCTGTCTTTTTCGTGTGTAAAGCCACACCTGTCTAAAAACATAATTCTTTAA (2002)

TCATTTGCCCTCTTTTCTCTGTGCTTCAATTAATAAAAAATGGAAGATCTAA..... 20 .....AA (2078)



6. Nucleotide sequence of the cDNA of human serum albumin, said nucleotide sequence is as follows:

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1      10      20
asp ala his lys ser glu val ala ala his arg phe lys asp leu gly glu glu asn phe lys
GAT GCA CAC AAG AGT CAG GTT GCT CAT CCG TTT AAA GAT TTG GCA GAA GAA AAT TTC AAA (170)

30      40      50
ala leu val leu ile ala phe ala gln tyr leu gln gln cys pro phe glu asp his val lys leu val asn glu val thr alu phe ala
GCC TTG GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT GAA GAT CAT GTA AAA TTA GTG AAT GAA GTA ACT CAA TTT GCA (260)

60      70      80
lys thr cys val ala asp glu ser ala glu asn cys asp lys ser leu his thr leu phe gly asp lys leu cys thr val ala thr leu
AAA ACA TGT GTT GCT CAT GAG TCA GCT GAA AAT TGT GAC AAA TCA CTT CAT ACC CTT TTT CCA GAC AAA TTA TGC ACA GTT GCA ACT CTT (350)

90      100      110
arg glu thr tyr gly glu met ala asp cys cys ala lys gln glu pro gly arg asn glu cys phe leu gln his lys asp asp asn pro
CGT GAA ACC TAT GGT CAA ATG GCT GAC TGC TGT GCA AAA CAA GAA CCT GGG ACA AAT GAA TGC TTC TTA CAC AAA GAT GAC AAC CCA (440)

120      130      140
asn leu pro arg leu val arg pro glu val asp val met cys thr ala phe his asp asn glu thr phe leu lys lys tyr leu try
AAC CTC CCC CCA TTG GTG ACA CCA GAG GTT GAT GTG ATG TGC ACT GCT TTT CAT GAC AAT GAA GAG ACA TTY TTG AAA AAA TAC TTA TAT (530)

150      160      168 169 170
glu ile ala arg arg his pro tyr phe tyr ala pro glu leu leu phe phe ala lys arg tyr lys ala ala phe thr glu cys cys gln
CAA ATT GCC ACA ACA CAT CCT TAC TTT TAT GCC CCG GAA CTC CTT TTC TTT GCT AAA AGG TAT AAA GCT GCT TTY ACA GAA TGT TGC CAA (620)

171      177      180      190      200
ala ala asp lys ala ala cys leu leu pro lys leu asp glu leu arg asp glu gly lys ala ser ser ala lys gln arg leu lys cys
GCT GCT GAT AAA GCT GCC TGC CTG TTG CCA AAG CTC GAT GAA CTT CGG GAT GAA GGG AAG GCT TCG TCT GCC AAA CAG ACA CTC AAG TGT (710)

201      210      220      230
ala ser leu gln lys phe gly glu arg ala phe lys ala trp ala val ala arg leu ser gln arg phe pro lys ala ala phe ala glu
CCC AGT CTC CAA AAA TTT CGA GAA ACA GCT TTC AAA CCA TGG GCA GTA GCT CCC CTG ACC CAG ACA TTT CCC AAA CCT GAG TTT GCA CAA (800)

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231 240 245 246 250 253 260  
 val ser lys leu val thr asp leu thr lys val his thr glu cys his gly asp leu leu glu cys ala asp asp ala asp leu  
 CTT TCC AAG TTA GTG ACA CAT CTT ACC AAA GTC CAC ACG GAA TCC TGC CAT GCA CAT CTG CTT GAA TGT CCT CAT CAC ACG CCG CAC CTT (890)

261 265 270 278 279 280 289 290  
 ala lys tyr ile cys glu asn gln asp ser ile ser ser lys leu lys glu cys qiu lys pro leu leu glu lys ser his cys ile  
 GCC AAG TAT ATC TGT TGT CAA AAT CAA CAT TCG ATC TCC AGT AAA CTG AAG GAA TCC TGT CAA AAA CCT CTG TTG GAA AAA TCT CAC TCC ATT (980)

291 300 310 316 320  
 ala glu val glu asn asp glu met pro ala asp leu pro ser leu ala ala asp phe val glu ser lys asp val cys lys asn tyr ala  
 GCC CAA GTG CAA AAT CAT CAG ATG CCT CCT CAC TTG CCT TCA TTA CCT CCT CAT TTT GTT CAA AGT AAG CAT GTT TGC AAA AAC TAT CCT (1070)

321 330 340 350  
 glu ala lys asp val phe leu gly met phe leu tyr glu tyr ala arg arg his pro asp tyr ser val val leu leu leu arg leu ala  
 CAG CCA AAG CAT GTC TTC TGC GCC ATG TTT TTG TAT GAA TAT GCA AGA ACG CAT CCT CAT TAC TCT GTC GTG CTG CAG CTT GCC (1160)

351 360 361 369 370 380  
 lys thr tyr glu thr thr leu glu lys cys cys ala ala asp pro his glu cys tyr ala lys val phe asp glu phe lys pro leu  
 AAG ACA TAT CAA ACC ACT CTA CAG AAG TGC TGT GCC CCT GCA CAT CCT CAA TCC TAT GCC AAA GTG TTC GAT GAA TTT AAA CCT CCT (1250)

381 390 392 400 410  
 val glu glu pro gln asn leu ile lys gln asn cys glu leu phe glu qiu leu qiu tyr lys phe qiu asn ala leu leu val arg  
 GTG CAA CAG CCT CAG AAT TTA ATC AAA CAA AAT TGT CAG CTT TTT GAG CAG CTT CCA CAG TAC AAA TTC CAG AAT CCG CTG TTA GTT CCT (1340)

411 420 430 437 438 440  
 tyr thr lys lys val pro gln val ser thr pro thr leu val glu val ser arg asn leu qiu lys val qiu ser lys cys cys lys his  
 TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT CCA ACT CTT GTA GAG GTC TCA ACA AAC CTA GCA AAA GTG GGC ACG AAA TGT TGT AAA CAT (1430)

441 448 450 460 461 470  
 pro glu ala lys arg met pro cys ala glu asp tyr leu ser val val leu asn gln leu cys val leu his qiu lys thr pro val ser  
 CCT CAA GCA AAA ACA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CAT AAC CAG TTA TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT (1520)

471 476 477 480 490 500  
 asp arg val thr lys cys cys thr glu ser leu val asn arg arg pro cys phe ser ala leu glu val asp qiu thr tyr val pro lys  
 CAC ACA GTC ACC AAA TCC TCC ACA GAA TCC TTG GTG AAC ACG CCA CCA TCC TTT TCA CCT CTG GAA GTC GAT GAA ACA TAC GTT CCC AAA (1610)

501 510 514 520 530  
 glu phe asn ala glu thr phe thr phe his ala asp ile cys thr leu ser qiu lys glu arg qiu ile lys lys qiu thr ala leu val  
 CAG TTT AAT GCT CAA ACA TTC ACC TTC CAT GCA GAT ATA TCC ACA CTT TCT CAG AAG GAG ACA CAA ATC AAG AAA CAA ACT CCA CTT GTT (1700)

ter ter

7. Nucleotide sequence coding for the prepeptide of human serum albumin, said nucleotide sequence is as follows:

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GCCTTTCTCTCTGTCACCCCCACACCCCTTTGCCACA ATG AAG TGG GTA ACC TTT ATT TCC CTT CTT TTT CTC TTT ACC (30)

-18 Met lys trp val tlu phe ile ser leu leu phe leu phe ser

p r o -10

-1 -6 p r o -1

ser ala tyr ser arg gly val phe arg arg  
TCC GCT TAT TCC ACC GGT GTC TTT CCT CGA

35	-6	p	r	o	-1	i	10	20	5																							
	arg	gly	val	phe	arg	arg	asp	ala	his	lys	ser	glu	val	ala	his	arg	phe	lys	asp	leu	oly	qlu	qlu	asn	phe	lys	120					
	ACG	GGT	GTG	TTT	CGT	CCA	CAT	CCA	CAC	CAC	AGT	CAG	GTT	GCT	CAT	CGG	TTT	AAA	CAT	TTG	GCA	GAA	GAA	AAT	TTC	AAA	(170)					
21	ala	leu	val	leu	lle	ala	phe	ala	gln	tyr	leu	gln	gln	oys	pro	phe	qlu	asp	his	val	lys	leu	val	asn	qlu	val	thr	qlu	phe	ala	50	
	CCC	TTG	GTG	TTG	ATT	GCC	TTT	GCT	CAG	TAT	CTT	CAG	CAG	TGT	CCA	TTT	GAA	CAT	GTA	AAA	TTA	GTG	AAT	GAA	GTA	ACT	CAA	TTT	GCA	(260)		
51	lys	thr	oys	val	ala	asp	glu	ser	ala	glu	asn	oys	asp	lys	ser	leu	his	thr	leu	phe	gly	asp	lys	leu	oys	thr	val	ala	thr	leu	80	
	AAA	ACA	TGT	GTT	GCT	CAT	CAG	TCA	GCT	GAA	AAT	TGT	GAC	AAA	TCA	CTT	CAT	ACC	CTT	TTT	CCA	GAC	AAA	TTA	TGC	ACA	GTT	GCA	ACT	CTT	(350)	
81	arg	glu	thr	tyr	gly	glu	met	ala	asp	oys	oys	ala	lys	gln	glu	pro	gly	arg	asn	qlu	oys	phe	leu	qln	his	lys	asp	asp	asn	pro	110	
	CGT	GAA	ACC	TAT	CGT	GAA	ATG	GCT	GAC	TGC	TGT	GCA	AAA	CAA	GAA	CCT	GGG	ACA	AAT	CAA	TGC	TTC	TTG	CAA	CAC	AAA	GAT	GAC	AAC	CCA	(440)	
111	asn	leu	pro	arg	leu	val	arg	pro	glu	val	asp	val	met	cys	thr	ala	phe	his	asp	asn	qlu	qlu	thr	phe	leu	lys	lys	tyr	leu	try	140	
	AAC	CTC	CCC	CCA	TTG	GTG	ACA	CCA	CAG	GTT	GAT	GTG	ATG	TGC	ACT	GCT	TTT	CAT	GAC	AAT	GAA	CAG	ACA	TTT	TTG	AAA	AAA	TAC	TTA	TAT	(330)	
141	glu	lle	ala	arg	his	pro	tyr	phe	tyr	ala	pro	glu	leu	leu	phe	phe	ala	lys	arg	tyr	lys	ala	ala	phe	thr	qlu	oys	cys	qln	168	169	170
	GAA	ATT	GCC	ACA	ACA	CAT	CCT	TAC	TTT	TAT	GCC	CCG	GAA	CTC	CIT	TTT	GCT	AAA	AGG	TAT	AAA	GCT	GCT	TTT	ACA	GAA	TGT	TGC	CAA	(620)		
171	ala	ala	asp	lys	ala	ala	oys	leu	leu	pro	lys	leu	asp	glu	leu	arg	asp	qlu	gly	lys	ala	ser	ser	ala	lys	qln	arg	leu	lys	cys	200	
	GCT	GCT	CAT	AAA	GCT	GCC	TGC	CTG	TTG	CCA	AGC	CTC	GAT	GAA	CTT	CCG	CAT	GAA	GGC	AGC	GCT	TGC	TGT	CCC	AAA	CAG	ACA	CTC	AAG	TGT	(710)	
201	ala	ser	leu	gln	lys	phe	gly	glu	arg	ala	phe	lys	ala	trp	ala	val	ala	arg	leu	ser	gln	arg	phe	pro	lys	ala	qlu	phe	ala	qlu	230	
	ACC	ACT	CTC	CAA	AAA	TTT	GCA	CAA	ACA	GCT	TTT	AAA	GTA	TGG	GTA	GTA	GCT	CTG	AGC	CAG	ACA	TTT	CCC	AAA	GCT	GAG	TTT	GCA	GAA	(300)		

231 val ser lys leu val thr asp leu thr lys val his thr glu cys cys his gly asp leu leu glu cys ala asp asp arg ala asp leu 260  
GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA GTC CAC ACC GAA TCC TCC CAT GCA CAT CTG CTT GAA TGT GCT CAT CAC AGC GCG CAC CTT (890)

261 ala lys tyr ile cys glu asn gln asp ser ile ser ser lys leu lys glu cys qly lys pro leu leu glu lys ser his cys ile 289 290  
GCC AAG TAT ATC TGT GAA AAT CAA GAT TCG ATC TCC ACT AAA CTG AAG GAA TCC TGT GAA AAA CCT CTG TTG GAA AAA TCC CAC TGC ATT (980)

291 ala glu val glu asn asp glu met pro ala asp leu pro ser leu ala ala asp phe val glu ser lys asp val cys lys asn tyr ala 320  
GCC GAA GTG GAA AAT CAT CAG ATG CCT GCT CAC TTG CCT TCA TTA CCT GCT CAT TTT GTT GAA AGT AAG GAT GTT TGC AAA AAC TAT CTT (1070)

321 glu ala lys asp val phe leu gly met phe leu tyr glu tyr ala arg arg his pro asp tyr ser val val leu leu leu arg leu ala 350  
GAG GCA AAG CAT GTC TTC TTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT TAC TCT GTC GTG CTG CTT GCA CTT GCC (1160)

351 lys thr tyr glu thr thr leu glu lys cys cys ala ala asp pro his qly cys tyr ala lys val phe asp glu phe lys pro leu 380  
AAG ACA TAT GAA ACC ACT CTA GAG AAG TCC TGT GCT GCT GCA CAT CCT CAT GAA TGC TAT GCC AAA GTG TTC CAT GAA TTT AAA CCT CTT (1250)

381 val glu glu pro gln asn leu ile lys gln asn cys glu leu phe qly leu qly glu tyr lys phe gln asn ala leu leu val arg 410  
GTG GAA CAG CCT CAG AAT TTA ATC AAA CAA AAT TGT GAG CAG CTT TTT GAG CAG CTT GCA CAG TAC AAA TTC CAG AAT GCG CTG TTA GTT CTT (1340)

411 tyr thr lys lys val pro gln val ser thr pro thr leu val glu val ser arg asn leu qly lys val qly ser lys cys lys his 440  
TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT CCA ACT CTT GTA CAG GTC TCA AGA AAC CTA GCA AAA GTG GCG AGC AAA TGT TGT AAA CAT (1430)

441 pro glu ala lys arg met pro cys ala glu asp tyr leu ser val val leu asn gln leu cys val leu his qly lys thr pro val ser 470  
CCT GAA GCA AAA AGA ATG CCC TGT GCA GAA CAC TAT CTA TCC GTG GTC CTG AAC CAG TTA TGT GTC CAT CAG AAA ACC CCA GTA AGT (1520)

471 asp arg val thr lys cys cys thr glu ser leu val asn arg pro cys phe ser ala leu glu val asp qly thr tyr val pro lys 500  
GAC AGA GTC ACC AAA TGC TGC ACA GAA TCC TTG GTG AAC AGG CGA CCA TCC TTT TCA GCT CTG GAA GTC CAT GAA ACA TAC GTT CCC AAA (1610)

501 glu phe asn ala glu thr phe thr phe his ala asp ile cys thr leu ser glu lys qly arg qln ile lys lys qln thr ala leu val 530  
GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TCC ACA CTT TCT GAG AAG CAG ACA CAA ATC AAG AAA CAA ACT GCA CTT GTT (1700)

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531 540 550 558 559 560  
glu leu val lys his lys pro lys ala thr lys glu gln leu lys ala val met asp phe ala ala phe val glu lys oys oys lys  
GAG CTC GTG AAA CAC AAG CCC AAG GCA ACA AAA GAG CAA CAA CTG AAA CCT GTT ATG CAT CAT TTC GCT GCT TTT GTA GAG AAG TGC TGC AAG (1790)

561 567 570 580  
ala asp asp lys glu thr oys phe ala glu glu gln lys lys leu val ala ala ser gln ala ala leu gly leu ter  
GCT GAC CAT AAG GAG ACC TGC TTT GCC GAG GAG GAG GGT AAA AAA CTT GTT GCT GCA AGT CAA GCT GCC TTA GCC TTA TAA CATCACATTAAAG (1883)

ter ter  
CATCTCAGCCTACCATGAGAAATAAGAGAAAGAAATGAAGATCAAAAGCTTATTCATCTGTTTTCCTGTTGCTGTAAGCCACACACCTGCTCTAAACATAAATTTCTTAA (2002)

TCATTTTGGCTCTTTCTGCTGCTTCAATTAAATAAAATAATGCAAGAAATCTAA..... 20 .....AA (2078)

9. Nucleotide sequence coding for the pre pro human serum albumin, said nucleotide sequence is as follows:

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10
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-16      -10
Met lys trp val tlu phe lle ser leu leu phe leu phe ser
ATG AAG TGG GTA ACC TTT ATT TCC CTT CTT TTT CTC TTT ACC (30)

-1      -6      p r o      -1      1
ser ala tyr ser arg gly val phe arg arg asp ala his lys ser glu val ala his arg phe lys asp leu oiy alu glu asn phe lys
TCC GCT TAT TCC ACG GGT GTG TTT CGT CGA GAT CCA CAC AAG AGT CAG GTT GCT CAT CGG TTT AAA GAT TTG CGA GAA AAT TTC AAA (170)

21      30      34      40      50
ala leu val leu lle ala phe ala gln tyr leu gln gln cys pro phe glu asp his val lys leu val asn glu val thr alu phe ala
CCC TTC GTG TTG ATT GCC TTT GCT CAG TAT CTT CAG CAG TGT CCA TTT CAA CAT CAT GTA AAA TTA GTG AAT GAA GTA ACT CAA TTT GCA (260)

51      53      60      62      70      75      80
lys thr cys val ala asp glu ser ala glu asn cys asp lys ser leu his thr leu phe gly asp lys leu cys thr val ala thr leu
AAA ACA TGT GTT CTT GAT CAG TCA GCT GAA AAT TGT CAG CAC AAA TCA CTT CAT ACC CTT TTT CGA CAC AAA TTA TCC ACA CTT GCA ACT CTT (350)

81      90      91      100      101
arg glu thr tyr gly glu met ala asp cys cys ala lys gln glu pro gly arg asn alu cys phe leu gln his lys asp asp asn pro
CGT GAA ACC TAT GGT GAA ATG GCT CAG TCC TGT CCA AAA CAA CAA CCT GCG ACA AAT GAA TCC TTC TTA CAC CAC AAA CAT CAC AAC CCA (440)

111      120      124      130      140
asn leu pro arg leu val arg pro glu val asp val met cys thr ala phe his asp asn alu glu thr phe leu lys lys tyr leu try
AAC CTC CCC CCA TTG GTG ACA CCA GAG GTT GAT GTG ATG TCC ACT CCT TTT CAT GAC AAT GAA CAG ACA TTT TTG AAA AAA TAC TTA TAT (530)

141      150      160      168      169      170
glu lle ala arg arg his pro tyr phe tyr ala pro glu leu leu phe phe ala lys arg tyr lys ala ala phe thr alu cys cys gln
CAA ATT GCC ACA ACA CAT CCT TAC TTT TAT TCC CCG CAA CTC CTT TTC TTT CCT AAA ACG TAT AAA GCT CCT TTT ACA GAA TGT TCC CAA (620)

171      177      180      190      200
ala ala asp lys ala ala cys leu leu pro lys leu asp glu leu arg asp alu gly lys ala ser ser ala lys aln arg leu lys cys
CCT GCT GAT AAA GCT GCC TCC TTG CCA AAG CTC GAT GAA CTT CCG GAT CAA GCG AAG CCT TCG TCT GCC AAA CAG ACA CTC AAG TGT (710)

201      210      220      230
ala ser leu gln lys phe gly glu arg ala phe lys ala trp ala val ala arg leu ser gln arg phe pro lys ala alu phe ala alu
CCC AGT CTC CAA AAA TTT CCA CAA ACA GCT TTC AAA CCA TGG CCA GTA CCT CCG CTC ACC CAG ACA TTT CCC AAA GCT CAG TTT GCA GAA (800)

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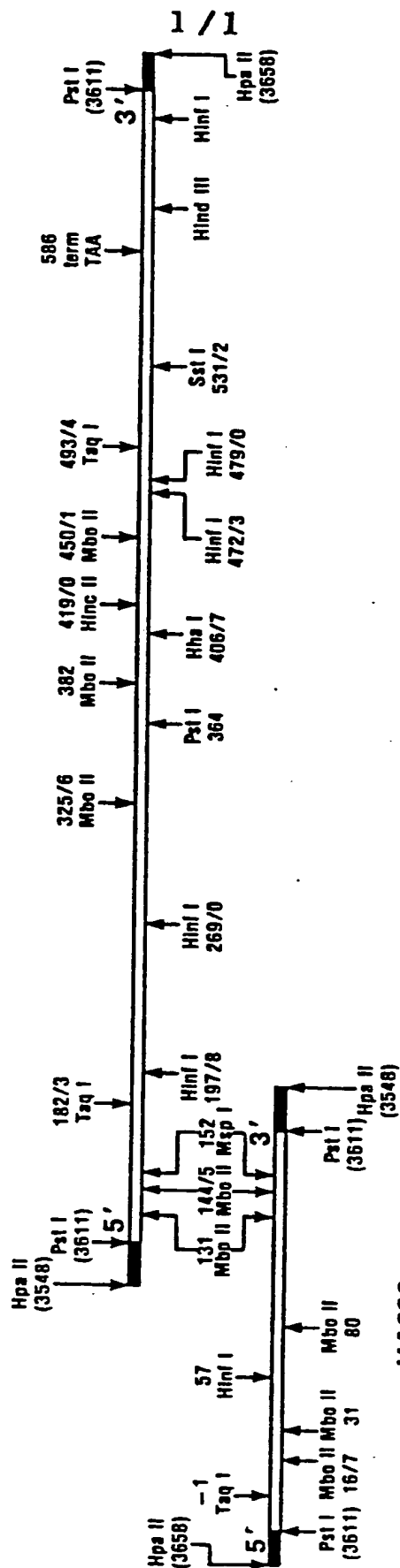
231 val ser lys leu val thr asp leu thr lys val his thr glu oys oys his gly asp leu leu glu oys ala asp asp arg ala asp leu 260  
 GTT TCC AAG TTA GTG ACA GAT CTT ACC AAA GTC CAC ACC GAA TCC TCC CAT GGA GAT CTG CTT GAA TGT GCT CAT CAC AGG CCG CAC CTT (A9N)  
  
 261 ala lys tyr lle oys glu asn gln asp ser lle ser ser lys leu lys glu oys oys glu lys pro leu leu glu lys ser his oys lle 289 290  
 GCC AAG TAT ATC TGT GAA AAT CAA GAT TCG ATC TCC AGT AAA CTG AAG GAA TCC TGT GAA AAA CCT CTG TTG CAA AAA TCT CAC TCC ATT (98N)  
  
 291 ala glu val glu asn asp glu met pro ala asp leu pro ser leu ala ala asp phe val glu ser lys asp val oys lys asn tyr ala 320  
 GCC GAA GTG GAA AAT GAT GAG ATG CCT GCT GAC TTG CCT TCA TTA CCT CAT TTT CTT GAA AGT AAG GAT GTT TCC AAA AAC TAT CTT (107N)  
  
 321 glu ala lys asp val phe leu gly met phe leu tyr glu tyr ala arg arg his pro asp tyr ser val val leu leu leu arg leu ala 350  
 GAG GCA AAG GAT GTC TTC TTG GGC ATG TTT TTG TAT GAA TAT GCA AGA AGG CAT CCT GAT TAC TCT GTC GTG CTG CCG ACA CTT CCC (116N)  
  
 351 lys thr tyr glu thr thr leu glu lys oys oys ala ala asp pro his glu oys tyr ala lys val phe asp glu phe lys pro leu 380  
 AAG ACA TAT GAA ACC ACT CTA GAG AAG TCC TGT GCT GCA GAT CCT CAT GAA TCC TAT GCC AAA GTG TTC CAT CAA TTT AAA CCT CCT (125N)  
  
 381 val glu glu pro gln asn leu lle lys gln asn oys glu leu phe glu leu phe glu tyr lys phe gln asn ala leu leu val arg 410  
 GTG GAA GAG CCT CAG AAT TTA ATC AAA CAA AAT TGT CAG CTT TTT GAG CAG CTT GCA GAG TAC AAA TTC CAG AAT CCG CTG TTA GTT CTT (134N)  
  
 411 tyr thr lys lys val pro gln val ser thr pro thr leu val glu val ser arg asn leu qly lys val qly ser lys oys oys lys his 440  
 TAC ACC AAG AAA GTA CCC CAA GTG TCA ACT CCA ACT CTT GTA GAG GTC TCA AGA AAC CTA GCA AAA GTG CCG AGC AAA TGT TGT AAA CAT (143N)  
  
 441 pro glu ala lys arg met pro oys ala glu asp tyr leu ser val val leu asn gln leu oys val leu his glu lys thr pro val ser 470  
 CCT GAA GCA AAA ACA ATG CCC TGT GCA GAA GAC TAT CTA TCC GTG GTC CTC AAC CAG TTA TGT GTG TTG CAT GAG AAA ACG CCA GTA AGT (152N)  
  
 471 asp arg val thr lys oys oys thr glu ser leu val asn arg arg pro oys phe ser ala leu glu val asp glu thr tyr val pro lys 500  
 CAC ACA GTC ACC AAA TCC TCC ACA GAA TCC TTG GTG AAC AGG CCA CCA TCC TTT TCA CCT CTG CAA GTC CAT GAA ACA TAC GTT CCC AAA (161N)  
  
 501 glu phe asn ala glu thr phe thr phe his ala asp lle oys thr leu ser glu lys glu arg gln lle lys lys gln thr ala leu val 530  
 GAG TTT AAT GCT GAA ACA TTC ACC TTC CAT GCA GAT ATA TCC ACA CTT TCT CAG AAG GAG ACA CAA ATC AAG AAA CAA ACT GCA CTT GTT (170N)



10. A nucleotide sequence according to any of claims 6 to 9, in essentially pure form.
11. A DNA transfer vector comprising a nucleotide sequence as defined in claim 5.
- 5 12. A DNA transfer vector according to claim 11, transferred to and replicated in a micro-organism.
13. A DNA transfer vector according to claim 12, which is a plasmid.
14. A DNA transfer vector according to claim 13, 10 wherein the plasmid is pBR322 or YEp6.
15. A process for preparing human serum albumin, which comprises culturing a micro-organism according to claim 5.
16. A DNA transfer vector according to any of 15 claims 12 to 14, or a process according to claim 15, wherein the micro-organism is a bacterium or yeast.
17. A vector or process according to claim 16, wherein the bacterium or yeast is E. coli or Saccharomyces cerevisiae.

# Restriction Endonuclease Map of Human Serum Albumin cDNA Clones

PHA36



PHA206

Kilobases

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